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The Development of the fruit of the Common Beans

THE DEVELOPMENT OF THE FRUIT OF THE COMMON BEAN UNDER DIFFERENT EXTERNAL CONDITIONS

BY

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY William Percival Snyder

ENTITLED The Development of the Fruit of the Common Bean under Different External Conditions

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science

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T A B L E O F C O N T E N T S

I INTRODUCTION

The object of this investigation is two-fold; first to follow the later phases of the development of the ovary and the ovule in Fucaceous fucoids; and second, to acquire the effects of different combinations of light and light on the structure and development of the ovule. No particular attention was paid to the earlier stages of development of the ovary, as these have been thoroughly studied by Guignard (1881), and Yamanouchi (1910). The present study begins with a mature ovule in which only one of the fertilization.

The egg of Pelvetia fastigiata, as that of other species, was made from sections of fixed material: Flemming's fluid, Bouin's fluid, Zenker's and Carnoy's fluid. No differences were found between the five varieties in regard to the staining of the cytoplasm and the nucleus. Guignard found that an alcohol mixture of equal parts of absolute alcohol, 95% alcohol and glacial acetic acid was the best fixative for the egg-cell. The first division of the oosphere is followed by successive nuclear divisions of the egg-cell. The oosphere are formed by transverse divisions of the egg-sphere cell. The wall of the two resulting cells was not removed, and a line was drawn between the nuclei as they appeared. The egg-sphere cells degenerate, however, as the young cell enlarges to form the oosphere. The antheridia, which also become separated from each other by their cross walls, are spheroidal. The flowing preserved full a complete fill from sporeling was from [illegible] by the syringe.

In the oosphere of Pelvetia fastigiata, the first division of the fertilized egg is transverse. The nuclear cell undergoes a division that

division, so that a pro-embryo of three cells is formed, of which the lower cell develops into the embryo, and the other two form the suspensor. A filamentous suspensor is formed by cells in Phaseolus, and with the basal cells considerably swollen. According to Brown, the suspensor is cut off when the embryo proper consists of about sixteen cells. Guignard found that the suspensor does not degenerate until after the differentiation of the cotyledons. From the time of the first appearance of the cotyledons, the suspensor does not increase in volume, except for the swelling of the cells in the upper part.

In the development of the endosperm, Guignard observed that the polar nuclei approach each other and begin to fuse before fertilization of the egg. On the other hand, [illegible] found that their fusion is not completed until after fertilization. The first two divisions of the endosperm nucleus [illegible] give four nuclei, of which two place themselves on either side of the embryo. The nuclei produced by subsequent divisions arrange themselves in the peripheral region of the embryo-sac. The divisions of the endosperm nuclei may be either simultaneous, or completely independent of each other. Endosperm cells are formed in the region immediately about the embryo, but are later absorbed.

In studying Vicia faba, [illegible] disagreed with Guignard in that he found a small amount of endosperm remaining unabsorbed in the mature seed.

II MATERIALS AND METHODS

The varieties used for the development studies were Burpee's New Kidney Wax and Extra Early Red Valentine. They were grown in field and greenhouse benches, at the plant physiology greenhouses, Botany Annex, University of Illinois, during the period from November

1918 to March, 1921, inclusive. There was a large loss among the earlier plantings due to faulty germination and to attacks of the red spider. The greenhouse temperature, observed in the afternoon, fluctuated as a rule between 70 and 85 degrees Fahrenheit. Specimens were taken of blossoms and of pods at different stages, ranging from those taken the first day after blossoming to nearly mature pods. Small pods were cut into half inch sections, and pods with a length of about two inches or more were cut so as to leave one third in each section. The material was then placed in Flemming's fixture, and allowed to remain for twenty-four hours or longer, after which it was washed in 70 per cent alcohol, run through the higher grades and imbedded in paraffin. Sections for staining were cut from seven to ten microns in thickness, stained in Delafield's hematoxylin, cleared in oil of cedar or Cage's fluid, and mounted in balsam.

The Extra Early Red Valentine variety was used in the studies on the effect of external conditions on the development of the pod. For this purpose, individual pods, when about an inch in length, were enclosed in test tubes plugged with cotton. The test tubes were treated in six different ways, as follows: water placed in the bottom of the tube; fresh calcium chloride placed in the tube; tubes enclosed in black paper; tubes containing water enclosed in black paper; tubes containing calcium chloride enclosed in black paper; and tubes with no special treatment. The calcium chloride was renewed from time to time, in order to maintain its efficiency, and the tubes receiving no treatment were changed daily after the pods became large, because of the condensation of moisture on their walls. A further test of moisture conditions was obtained by covering two plants with large bell jars, and taking pods from these plants to compare with those

from adjacent plants not covered with cell jars. The air under the cell jars became saturated in a short time, as indicated by the copious condensation of water on their sides. The moisture conditions were such that pods could not be grown to complete maturity, as they began to rot when about grown. The leaves of the plants were attacked by fungi, and the plants so weakened that they stopped blooming in a very short time. Pods taken from these plants and from the kept tubes were treated and studied in the same way as those used for the developmental study. Notes were taken on the length of the pods when cut, the number of days they had been kept in test tubes, and their texture.

III. THE DEVELOPMENT OF THE SEED

As mentioned in the introduction, the present study began with a stage probably just before fertilization. The material for this stage was taken in the afternoon of the day on which the blossom opened. The synergids and fertilized egg are shown, surrounded by a solid mass of nucellus (Figs. 1 and 2). The peripheral region of the embryo sac is occupied by a tissue composed of large, thin walled cells, derived from the nucellus. A part of this nucellar tissue forms an upward projection from the chalazal end of the embryo sac, and apparently serves as a means for absorbing and transferring plant food to the growing embryo. Twenty-four hours later, fertilization has occurred, and the three-celled proembryo has developed (Fig. 3).

The earliest observed stage of the suspensor was found in sections taken from pods one inch in length. The suspensor at this time consisted of two tiers of three cells each, with the basal cells considerably smaller. Later four tiers of cells are developed, and

the number of cells per tier is increased. The size of the cells decreases from the basal cells towards the embryo, so that in later stages the suspensor seems to merge into the pistils, without any definite line of separation. After the ovule has grown one-eighth inch in diameter, there is very little increase in size of the suspensor, and it persists without disintegration in the mature specimens observed. When the suspensor consists of six cells, the condition is the four shown in Fig.9, and shows a distinctly differentiated sphaeroid in the distal region. The cotyledons appear a little later and may be seen in cross section in Figs. 8,9,10 and 11. The plumule develops from the terminal portion of the hypocotyl shortly after the appearance of the cotyledons. The cells which are by this time the most may have been cut off by this time, and soon develop into a small protuberance at the end of the hypocotyl (Fig.10). The hypocotyl at first lies with its long axis approximately at right angles to that of the embryo sac, but by the time the embryo has filled the sac, the hypocotyl, with the suspensor, seems to curve inward, and in the mature seed lies close against the dorsal edges of the cotyledons.

In the earliest preparations, the cells of the inner integument adjoining the micropyle are very highly stained, and persist until late in the history of the embryo-sac, when they are finally absorbed. The earliest stages also show three or four cell layers of the inner part of the seed coat to be more deeply stained than the surrounding tissue, and very soon these cells begin to break down, so that as the embryo-sac enlarges it is constantly surrounded by a region of disorganizing tissue. This region comes into contact with the suspensor and integument tissue at the micropylar end of the sac,

and with the nucellar tissue at the chalazal end. Elsewhere the epidermal wall of the sac remains during the earlier stages, and ultimately fails also to break down. Coulter and Chamberlain (2), page 100, state that this method of absorption of adjacent tissue by the endosperm is the most common means of obtaining nutrition. The nucellar tissue gradually diminishes in size as the sac enlarges, soon becoming a pear-shaped structure, and by the time the embryo has filled the sac, there is also only a very thin layer next to the funiculus. This tissue is intimately associated with the vascular tissue of the funiculus, in some preparations being apparently merged with it, and undoubtedly also in transferring nutritive supplies to the growing embryo.

The endosperm develops rapidly, and soon forms a layer extending around the periphery of the sac, with a few strands connecting the peripheral layer and the portion surrounding the embryo. The endosperm surrounds the embryo, extending down to the suspensor. In this portion of the endosperm particularly, and to a lesser extent in the other regions of the sac, endosperm cells are found, not forming a continuous tissue, but separately embedded in the general mass of cytoplasm. Free nuclei are also distributed through the endosperm tissue. As perfectly mature seeds were not used in the present study, a thin layer of endosperm was left even in full grown seeds, between the tips of the cotyledons and the seed coat, and also in the micropylar cavity, around the extremity of the radicle.

IV THE EFFECT OF EXTERNAL CONDITIONS ON FOOD AND SIZE

In these studies, pods in approximately equal stages of development, and sections from corresponding parts of the seed were taken for comparison. In all cases the seed was nearly or quite full

grown. The growth rate of the pods seemed quite similar in both all conditions, and it was impossible to determine whether or not the rate was influenced by the different treatments. It would have been necessary to grow a much larger number of pods than was done in this experiment, in order to detect any such influence. The texture of the pods was tested by cutting through them with the razor blade. Pods grown in moist air seemed to be softer and less brittle than those grown in dry air, and due to the reduction of woody tissue the string was more easily cut.

The wall of the pod, as seen in cross section, shows the following structures: an external row of epidermal cells, beneath which is a layer of palisade-like cells; next to these are two regions of thin walled parenchyma cells of which the outer layer is composed of large cells adapted to storage, while the inner layer is composed of smaller pithy cells, usually showing a progressive decrease in size towards the inner side of the pod wall. The innermost row of these cells usually has the appearance of an epidermis in the regular arrangement and rectangular shape of the cells. Between the outer and inner layers of parenchyma, there is an intermediate zone, usually consisting of a thin layer ofmeristematic cells. A number of small vascular bundles are scattered through the outer layer of parenchyma. Just beneath the ventral and dorsal sutures, the pod wall is occupied by the two main vascular bundles, each with a prominent outer layer of sclerenchyma cells. Vascular tissue extends from the dorsal bundle to the seed, through the funicle.

The layers of the seed coat resemble those figured by Harz (6) for Soja hispida, with the exception of the fourth layer, which is either absent or fused with the fifth layer. There is an outer layer of very long and narrow palisade cells, a second layer

consisting of a single row of very small, regular, thick walled cells, a third layer of parenchyma cells, containing the scattered vascular bundles, and an innermost layer of sclerenchyma tissue. In all specimens examined, traces of endosperm were found between the seed coat and cotyledons. It is possible that even these traces would have been absorbed in perfectly mature seeds.

A. Effects of Moist and Dry Air

Specimens grown in dishes containing calcium chloride were compared with specimens grown in dishes containing water. In pods grown in dry air, the meristematic layer of the pod wall was much thinner between the outer and inner epidermis, while in pods grown in moist air, this layer was considerably nearer to the inner epidermis, so that in these pods the outer layer of parenchyma was decidedly thicker than the inner layer. In the mature specimens the entire pod wallwas much thinner when grown in moist air. The following modifications were also observed in pods grown in moist air. There was only a slight decrease in the size of the cells of the inner parenchyma, in going from the middle to the inner side of the pod wall, whereas there was a very marked decrease in the size of pods grown in dry air. In pods grown in moist air, the epidermal cells were narrow, and tangentially elongated. There was almost a complete absence of epidermal hairs. The palisade-like cells of the seed coat were longer, while the cells of the third layer had shorter radial and longer tangential diameters. There was practically no difference in the thickness of the seed coat.

The pods grown in moist air had a thicker funiculus, which contained a greater proportion of the vascular tissue. The vascular bundles on the ventral side were larger, while the sclerenchyma

region of the dorsal side was longer and narrower than the ventral region in pods grown in dry air, and the cells had shorter radial diameters. In the case of the pods grown in darkness, the central sclerenchyma region was decidedly more extensive in the pods grown in moist air, while the dorsal sclerenchyma region was larger in those grown in dry air. The cells in the ventral region had greater radial diameters in the specimens grown in moist air.

D. Effects of Light and Darkness

Specimens grown in tubes covered with black paper were compared with specimens grown in uncovered tubes. In the case of the pods grown in darkness, the inner parenchyma layer of the pod wall was much narrower than the outer layer. The cells of the outer layer were rather less elongated, and the cells of the inner layer were smaller, and slightly more elongated tangentially, than in pods grown in light. The epidermal cells were very narrow radially. In the case of pods grown in dry air, the epidermal hairs were less developed in the specimens grown in darkness than in those grown in light. The palisade cells were less distinctly differentiated from the parenchyma cells, when grown in darkness, and had greater tangential diameters. The cells of the third layer of the seed coat were shorter. The ventral region of sclerenchyma was larger and narrower, while the cells were wider. The cells of the dorsal sclerenchyma were smaller in one case, larger in another and in a third there was practically no difference. This seems to indicate a large individual variation. In like manner, the funiculus was much wider when grown in light and when no moisture treatment was given, while of the specimens grown in dry air, the one kept in darkness showed the wider funiculus.

C. Discussion.

The modifications brought about by the difference in atmospheric moisture [illegible] which affect the rate of transpiration, such as thickness of epidermis, presence or [illegible] development of epidermal hairs, and thickness of pod wall. The modifications that would increase the rate of transpiration in the moist atmosphere, and decrease the rate in the dry atmosphere. The greater radial elongation, under moist conditions, of the cells lying just beneath the epidermis, is not in agreement with the observations of Eberhardt (4) who, in working with Tussilago fulgens, found the leaf thickness to be increased in a dry atmosphere, due to the greater development of palisade tissue. However, what have been termed "palisade-like cells" in the present experiment, are not the typical palisade cells found in foliage leaves. It is very probable that the photosynthetic process is carried on by several deeper lying cell layers, if not by all of the outer region of parenchyma in the pod wall. The fact that this outer region was thicker under moist conditions, and that these cells serve for food storage, indicates that a greater amount of food is stored in the pod wall under such conditions. However, a large proportion of this food is doubtless transferred from other parts of the plant. The pods grown in darkness showed a considerable amount of starch in these cells. The greater thickness of the fun[illegible] facilitates the passage of water and dissolved food into the seed. An increase in the moisture conditions should be accompanied by less development of mechanical tissue, but the observations were very unsatisfactory in this respect. As was pointed out above, under II A, in the case the area of ventral sclerenchyma was larger, while that of the dorsal sclerenchyma was smaller, in a specimen grown in moist

air. In one instance two walls of the sclerenchyma fibres were thicker when grown in moist air. The tendency to the production of long, narrow cells under moist conditions is evident in the elongation of the parenchyma cells of the

According to Palladin (7), page 284, the structural changes involved in etiolation are chiefly due to the increased transpiration in darkness. Long tubes examined are similar to those which are brought about by excessive moisture. In the present case this fact is illustrated by the thinner epidermis, and, when a dry atmosphere supplies the conditions for the production of epidermal hairs, by the lessened development of these hairs; and also by the increased proportion of the outer layer of parenchyma in the pod wall. The parenchyma cells of the seed coat, however, were shortened in length-wise, whereas they were elongated in moist air. The usual decreased amount of palisade tissue in etiolated plants is paralleled by the fewer, and partially differentiated palisade-like cells of the pod wall. While a lessened development of strengthening tissue is indicated by the wider lumen of the cells of the ventral sclerenchyma, the discrepant results with respect to the dorsal sclerenchyma and the funiculus indicate that individual variation is here sufficient to obscure the differences due to the different treatments. The chief well marked differences brought out by the present study, are in the structural modifications which tend to augment the transpiration rate and to increase the proportion of storage tissue in the pod, and to some extent in the shape of the parenchyma cells of the seed coat.

V SUMMARY

Particular attention was paid only to the later stages of the development of the embryo. These show a filamentous suspensor, with the basal cells considerably swollen, which persists to the maturity of the seed, but increases very little in size after the very early stages. The cotyledons, followed shortly by the plumule, do not appear until some time after the differentiation of the epidermis. The cells of the root cap are developed with, or soon after the cotyledons. During the growth of the seed the hypocotyl arches curved inward until at maturity it lies in close contact with the cotyledons.

The nucellar tissue extends into the funiculus, but aids in transferring nutriment to the embryo. The endosperm envelops rapidly, surrounds the embryo, and also forms a layer extending around the periphery of the embryo sac. Both free nuclei and isolated cells occur in the endosperm. The nucellar tissue is gradually absorbed throughout the development of the embryo, and when the seed is nearly mature, only a thin layer of nucellus tissue, a little like of endosperm, remains about the funiculus. A thin layer of endosperm is also left in the micropylar cavity. Throughout the development of the embryo there is a lisi testifying layer of tissue three or four cells deep in the inner integument, and which ultimately envelops the epidermal wall of the embryo sac.

The growth of the pods in a very moist atmosphere, as compared with a very dry atmosphere caused the development of structural modifications of the pod wall favoring rapid transpiration, an increase in the percentage of mature tissue in the wall of the pod, increased thickness of the funiculus, and in the case of the seed coat, radial elongation of the palisade cells and partial elongation and of the parenchyma cells. The exclusion of light from the growing

pole had the same effect as excessive moisture in the atmosphere of the spikelets and the amount of storage tissue in the pod, and produced an increase in the radial diameter of the parenchyma cells of the seed coat. No definite or real relative proportion of vascular tissue developed under the different treatments indicated, but the results of the treatment were obscured by individual variation in this respect. However, tests made of a considerable number of pods by cutting with a razor blade, indicated a smaller development of woody tissue when the pods were grown in a moist atmosphere.

I wish to express my sincere appreciation to Dr. C.F. Watts, for suggesting the present study, and for much valuable advice and assistance.

EXPLANATION OF FIGURES

Figs.1 and 2.- Micropylar portion of ovary-sac before fertilization. e, synergids; e. sac, n, nucellar tissue; i, cells of inner integument.

Fig.3.- Longitudinal section of ovary-sac with four young ovules. n, nucellus; pro-embryo; i, region of young ovules; ii, cells of integument; h, hypocotyl.

Fig.4.- Ovary-sac, from the same specimen as Fig.3, showing nucellar tissue n.

Fig.5.- Young ovule from pod one inch in length. e, embryo-sac; h, tail of sac; r, differentiating cells; m, micropyle; und, endosperm; n, nucellar tissue; i, integument; i, differentiating cells of integument; ep, epidermis of ovule; f, funiculus; b, bract.

Fig.6.- Micropylar portion of embryo-sac of Fig.5. s, synergid cells; es, embryo; ep, differentiating epidermal cells; i, deeply stained cells of integument; und, endosperm cells.

Fig.7.- Hypocotyl and neighboring tissues in nearly full grown seed. The bending of the hypocotyl towards the cotyledons is not quite completed. h, hypocotyl; p, plumule; c, tissue of cotyledon; ep, epidermis; e, endosperm, of which the upper portion is removed layer by layer.

Figs. 8,9,10,11.- Cross sections of young ovule showing successive stages of development. In Fig.11, the hypocotyl has grown toward inward: i, integumentary tissue; e, epidermis of ovule; e', epidermis of embryo; m, micropyle; s, embryo-sac; c, cotyledons; h, hypocotyl; p, plumule; sp, suspensor cells; end, endosperm; v, vascular tissue; rc, cells of root cap; n, nucellar tissue.

Fig.12.- Cross section at base of hypocotyl of young seed. The hypocotyl has begun to bend toward the micropyle.

parallel to the edges of the cotyledons, and as appears in cross section. c, cotyledons; h, hypocotyl; r, radicle; s_1, s_2, and s_3, first, second and third layer of seed coat.

Figs. 13 and 14.- Comparison of seed coat structure grown in moist air (Fig.13) and dry air (Fig.14).

Figs. 15 and 16.- Comparison of seed coat structure grown in light (Fig.15) and darkness (Fig.16).

Figs. 17 to 20 inclusive.- Comparisons as before. Fig.17, grown in dry air; Fig.18, grown in dry air and darkness; Fig.19, grown in moist air; Fig.20, grown in moist air and darkness. In Figs. 18 to 20 inclusive, there is an outer row of palisade cells, a second row of small thick-walled cells, a layer of parenchyma, a layer of disorganized tissue, and lastly, a row of endosperm cells. v, vascular bundles.

Fig.21.- Cross section of pod wall, grown in light with no moisture treatment, showing a few cells of the different regions. e, epidermis; p_1, outer parenchyma layer; m, mesenchymatic region, p_2, inner parenchyma layer; e, inner epidermal cells.

Figs.22 and 23.- Comparison of wall grown in light (Fig.22) and darkness (Fig.23). v, vascular bundles.

Figs. 24 and 25.- Comparison of pod wall grown in moist air (Fig.24) and dry air (Fig.25). Note the proportions of the inner and outer layers of parenchyma. The regions are as shown on Fig.21.

Figs. 26 to 28 inclusive.- Comparison of both parenchyma regions and large vascular bundles of pod wall. Collenchyma cells are shown in each case, under a higher power than the upper part of the figure.

Fig.26, Ventral region, grown in darkness.

Fig.27- Ventral region, grown in light.

Fig.28- Ventral region, grown in moist air.

Fig.29- Ventral region, grown in dry air. s, sclerenchyma; v, vascular bundle.

Fig.30- Funicular and ventral region of [illegible] wall, grown in moist air. f, funicular; s, sclerenchyma; v, vascular bundles.

Fig.31- Funicular and ventral region of [illegible] wall, grown in dry air. f, funicular; s, sclerenchyma; v, vascular bundles.

Fig.32- Portion of epidermis grown in light, no visible treatment.

Fig.33- Portion of epidermis grown in dark, no visible treatment.

Fig.34- Portion of epidermis grown in dry air and darkness.

Fig.35- Portion of epidermis grown in dry air and light.

Fig.36- Portion of epidermis grown in dry air (young stage than Fig.35.)

Fig.37- Portion of epidermis grown in moist air (same stage as Fig.36).

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Fig 1.

Fig 2.

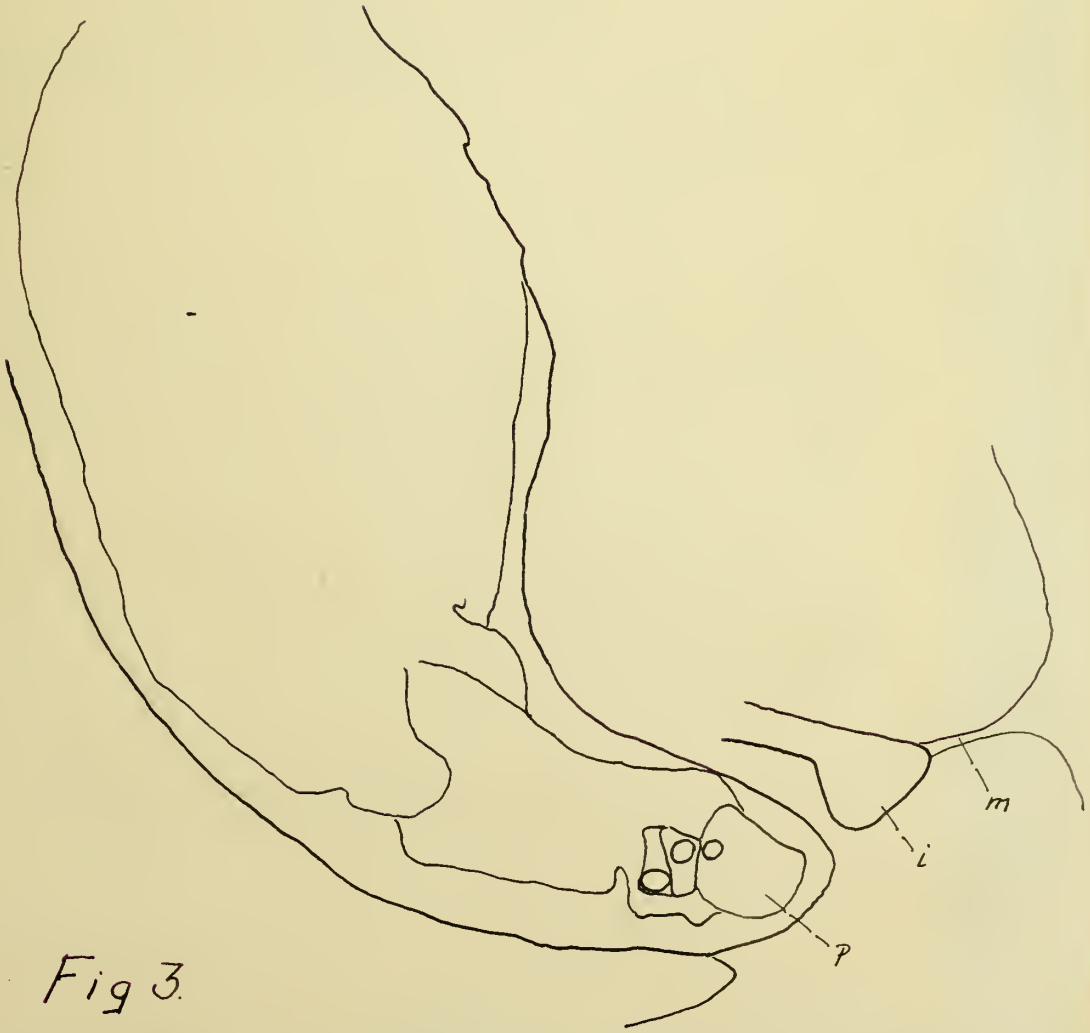


Fig 3.

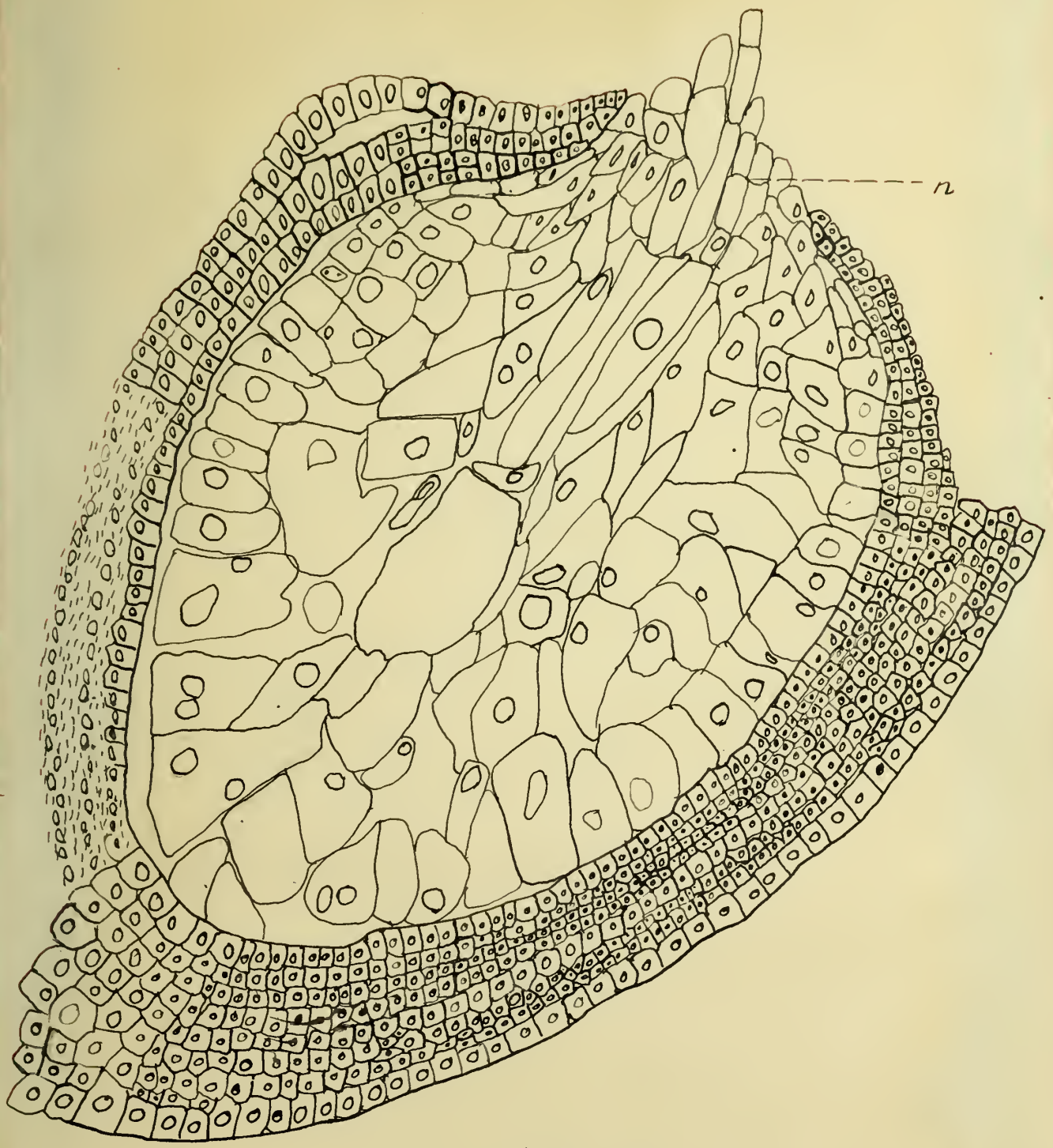


Fig. 4.

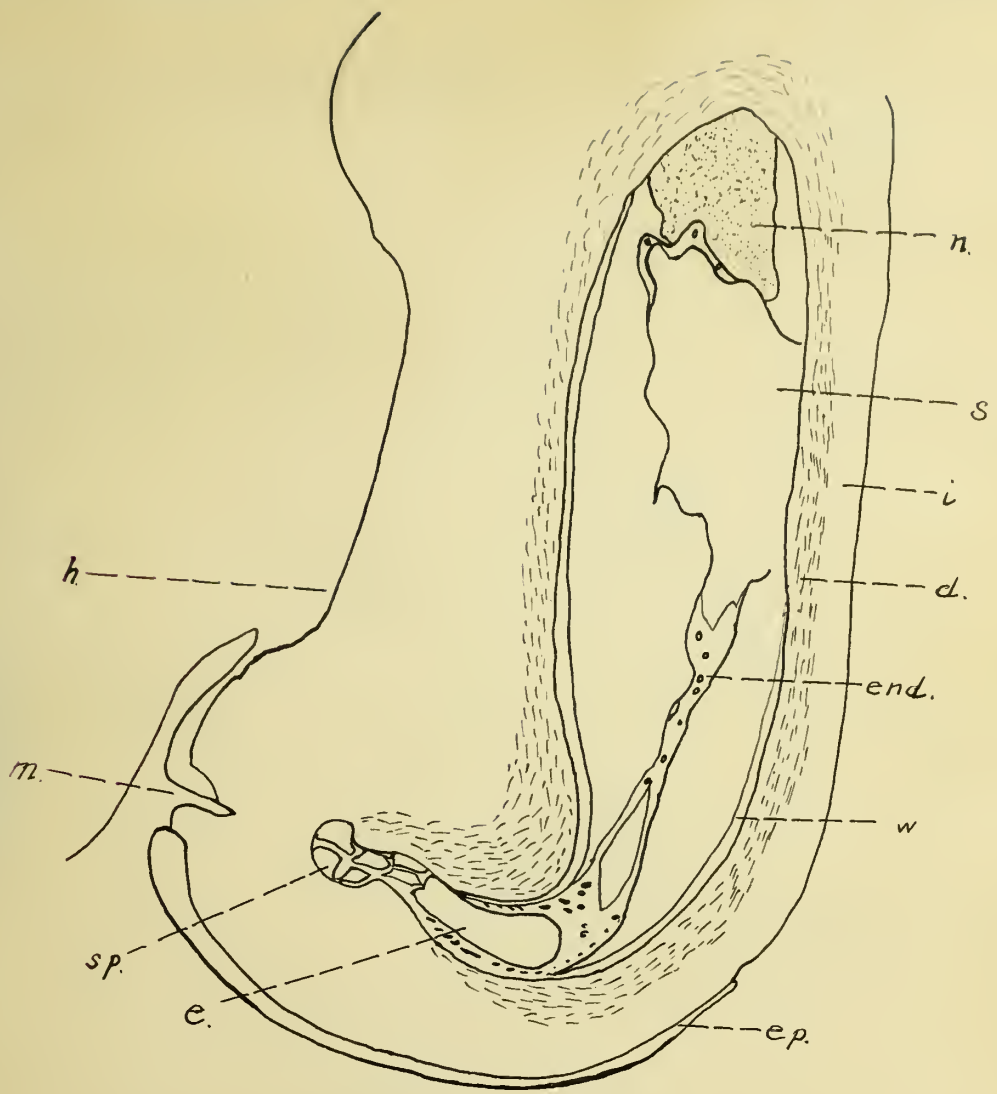


Fig. 5.

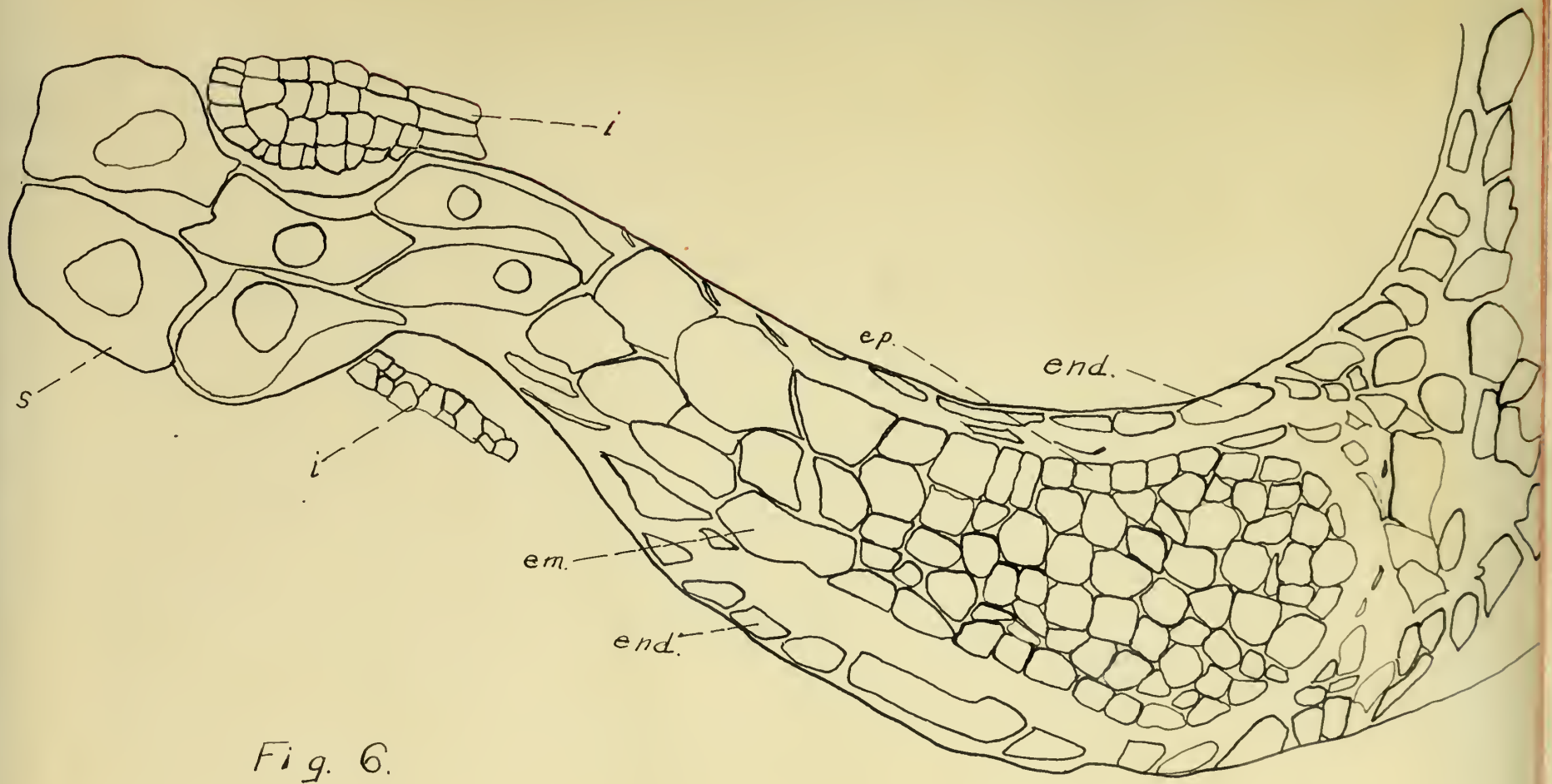


Fig. 6.

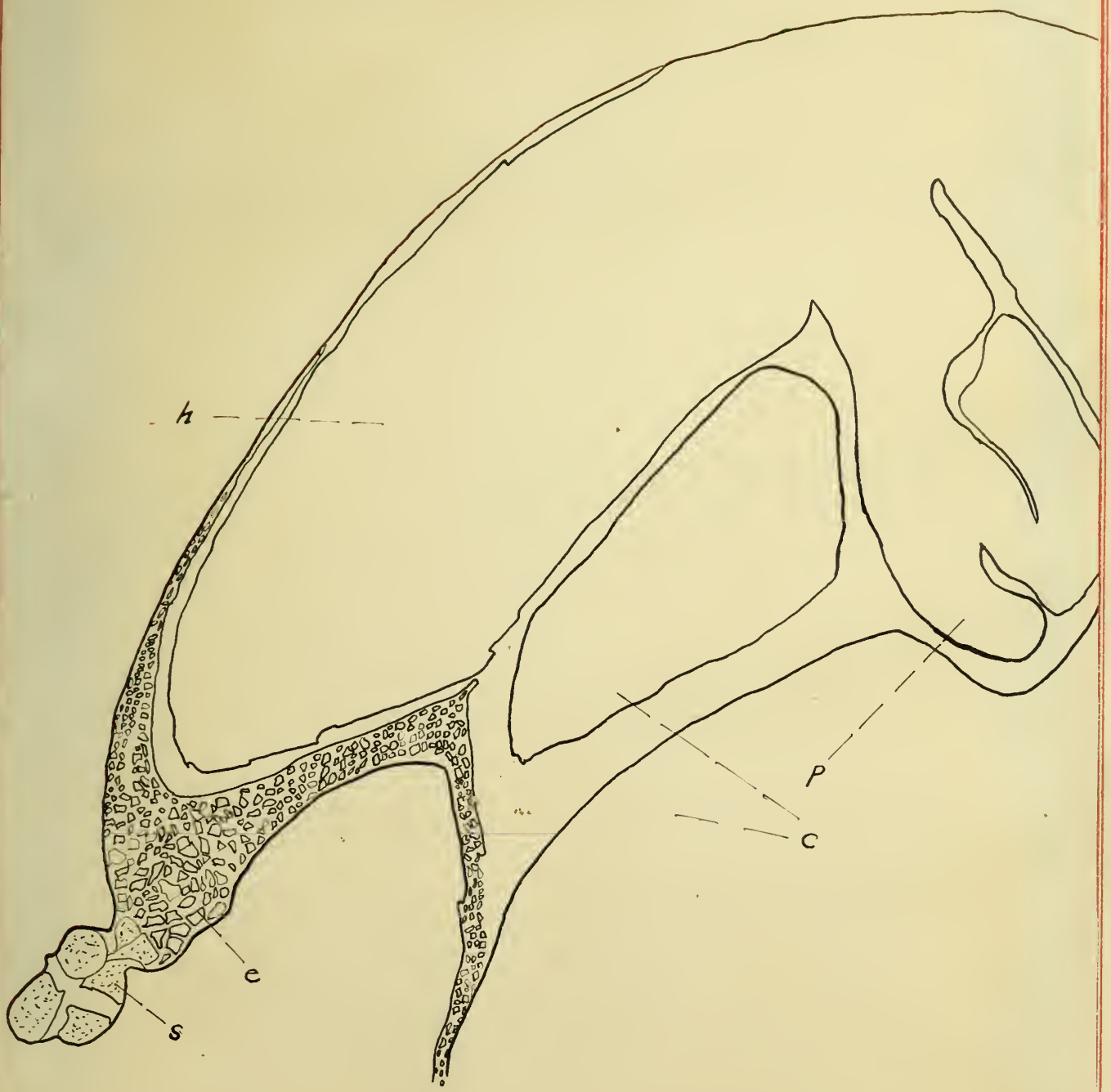


Fig. 7.

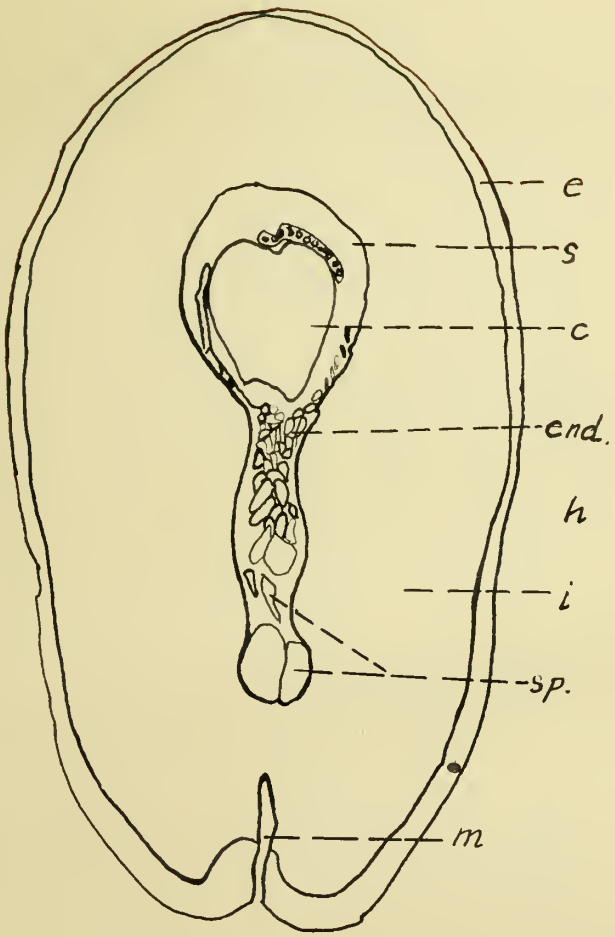


Fig. 8.

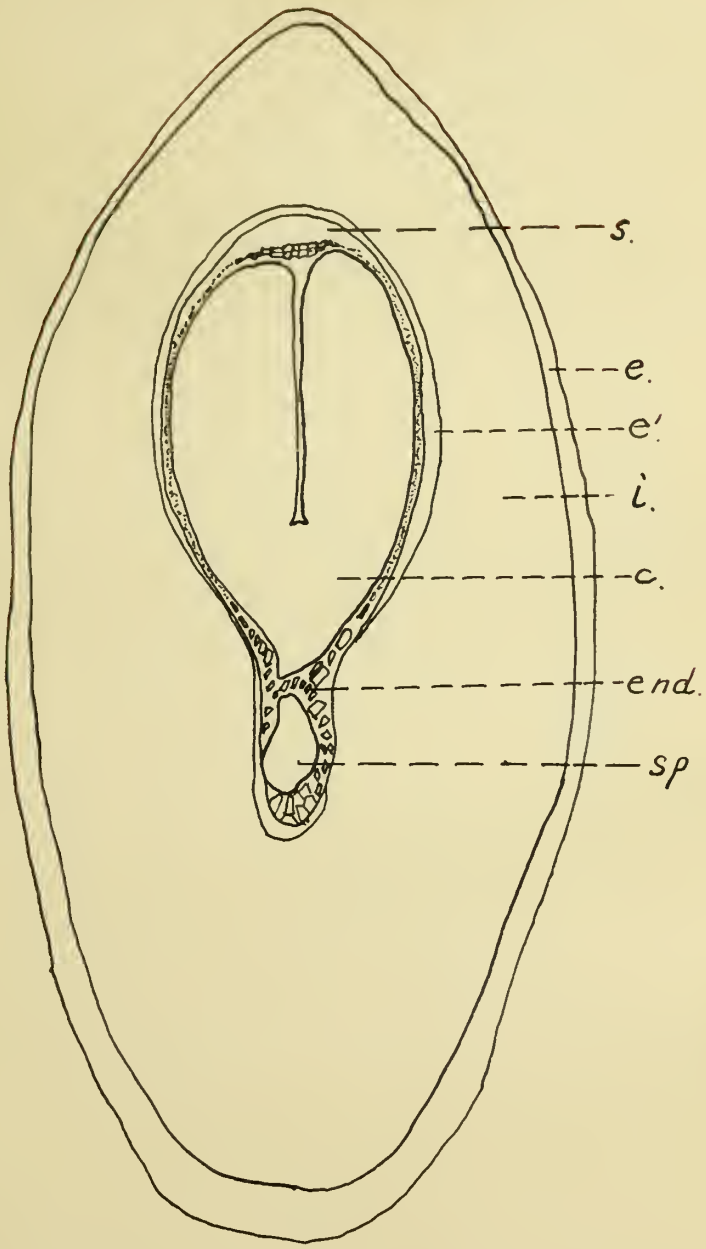


Fig. 9.

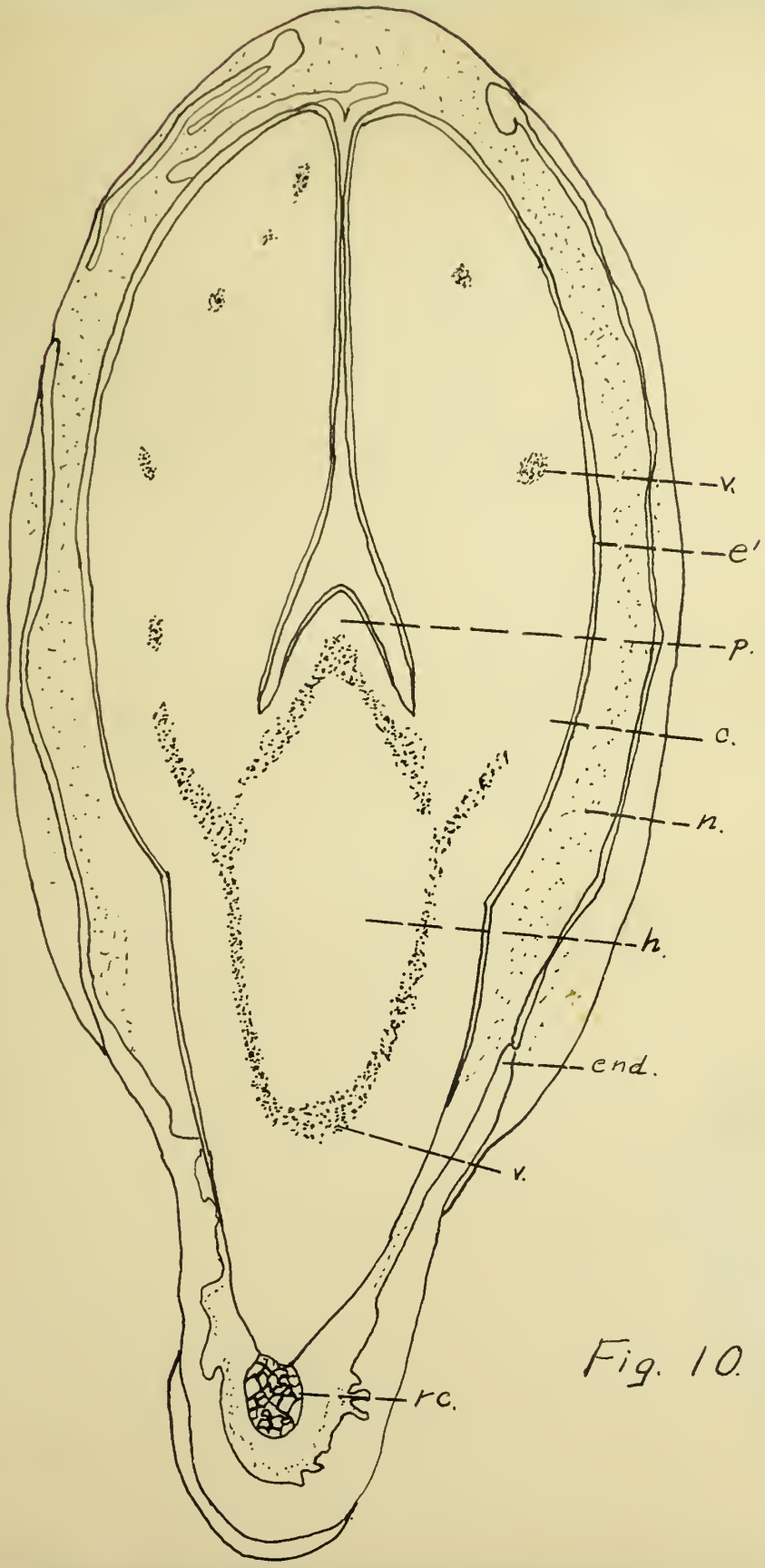


Fig. 10.

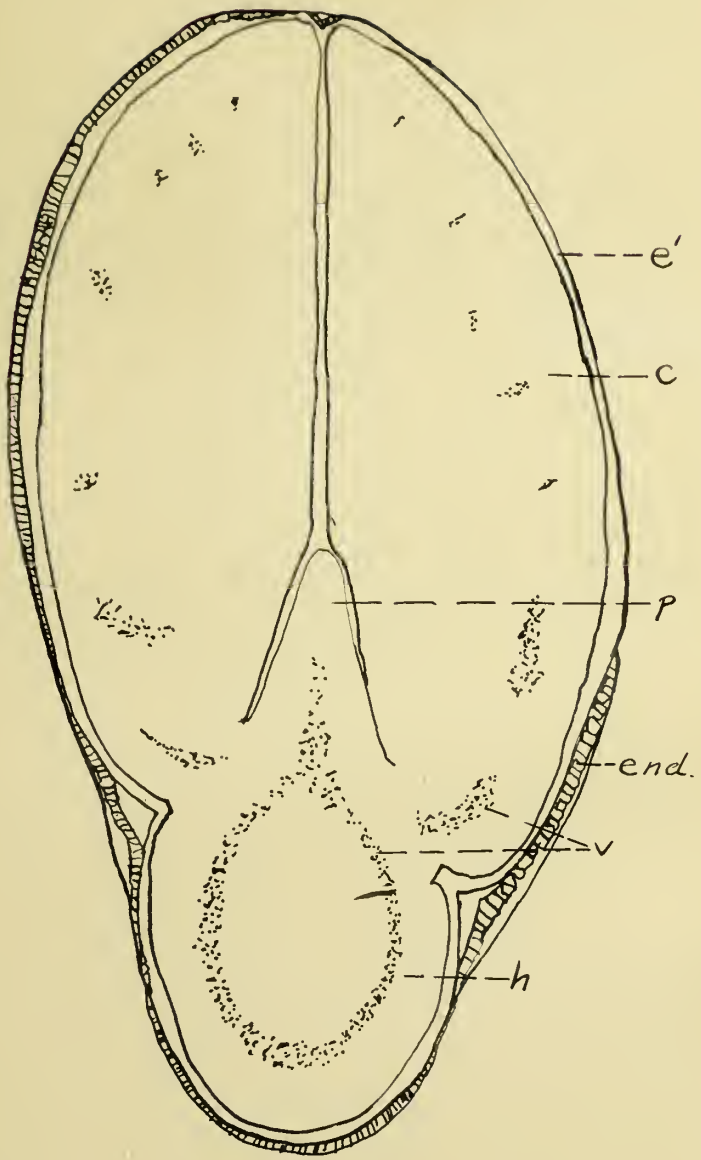


Fig. 11.

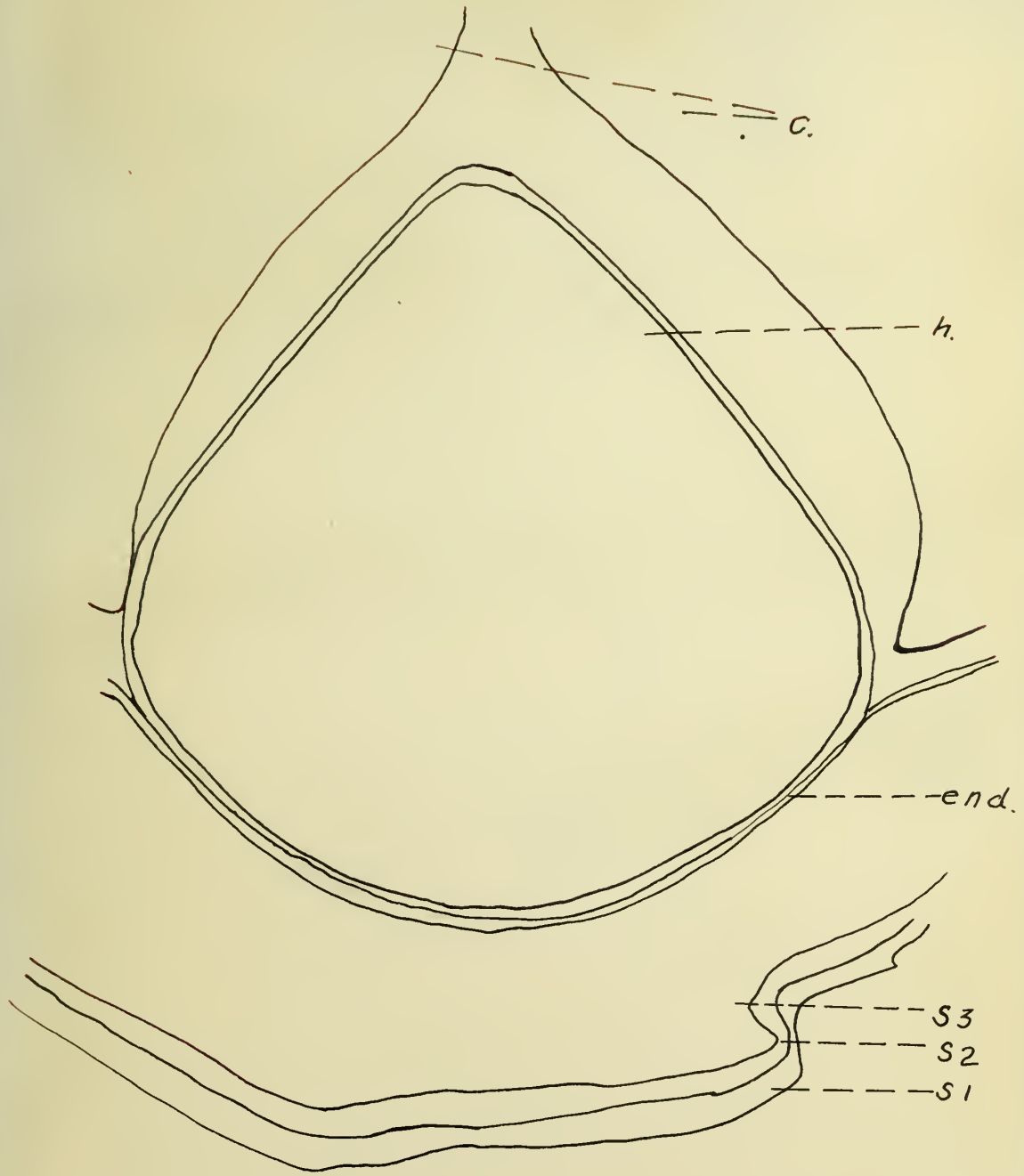
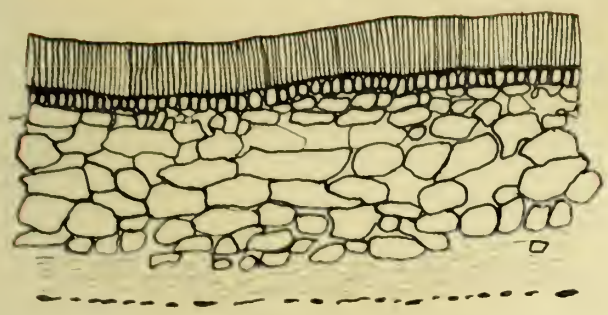


Fig. 12.



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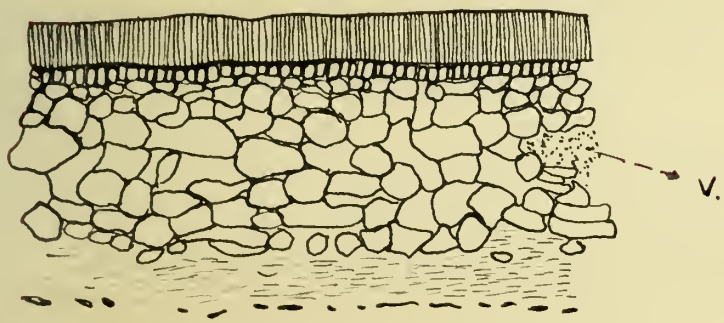

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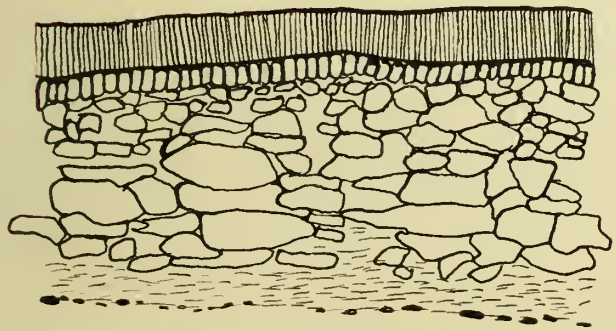

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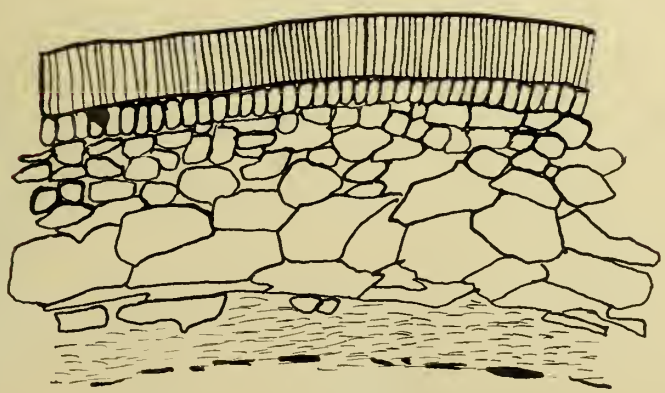

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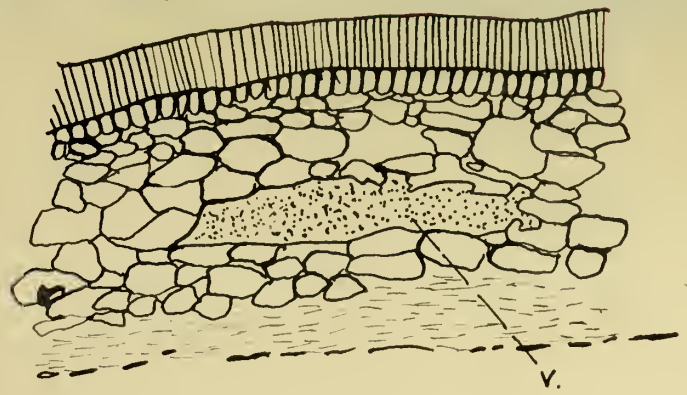

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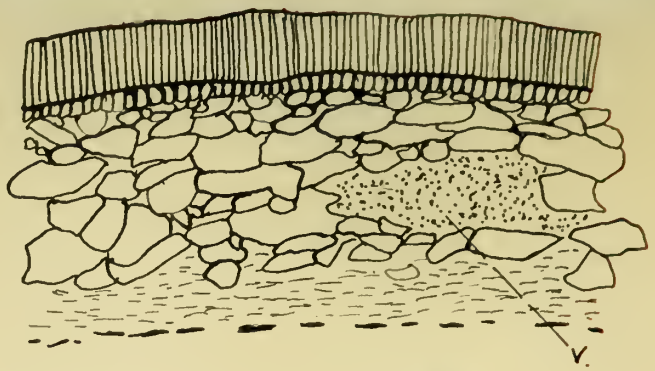

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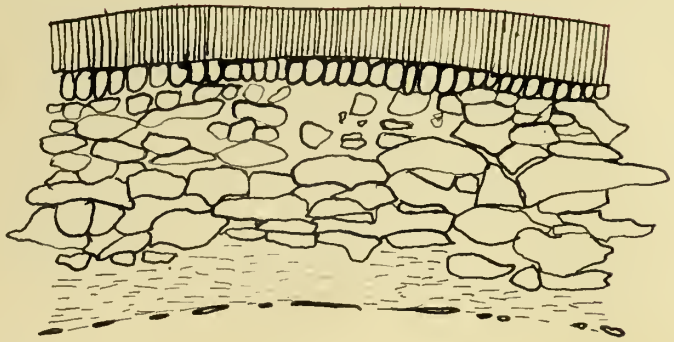

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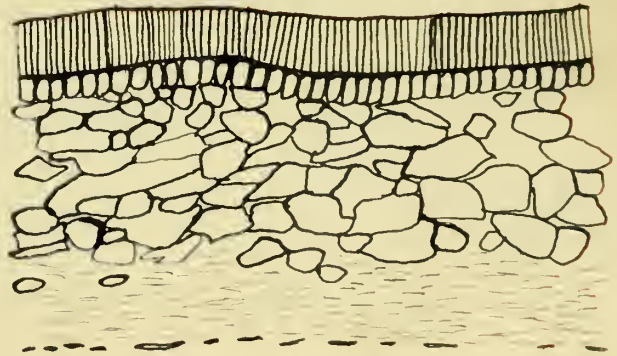

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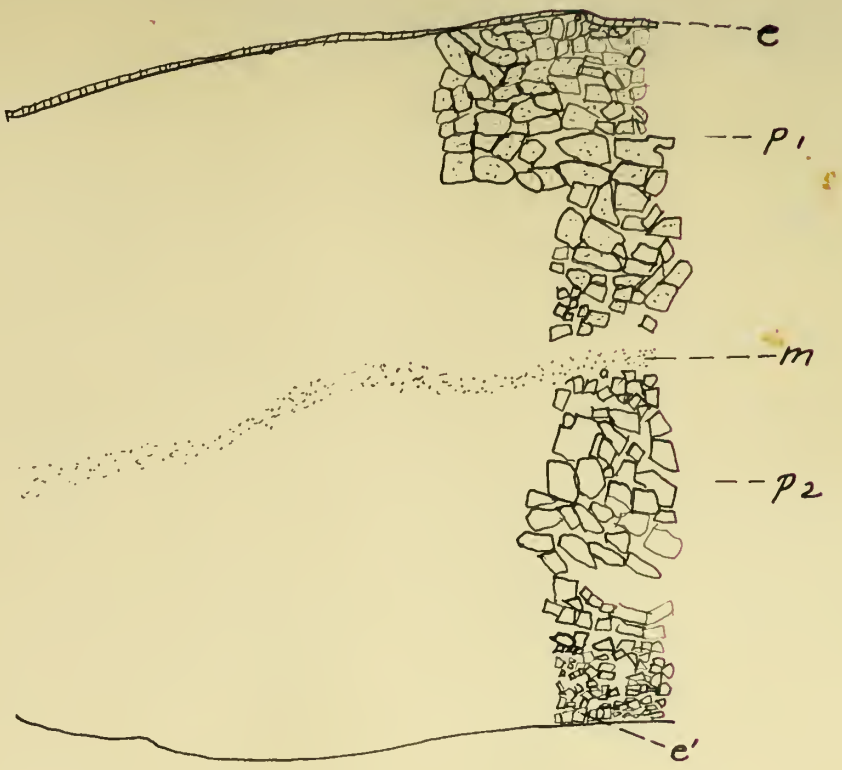

Fig. 21.

Fig. 22.

Fig. 23.

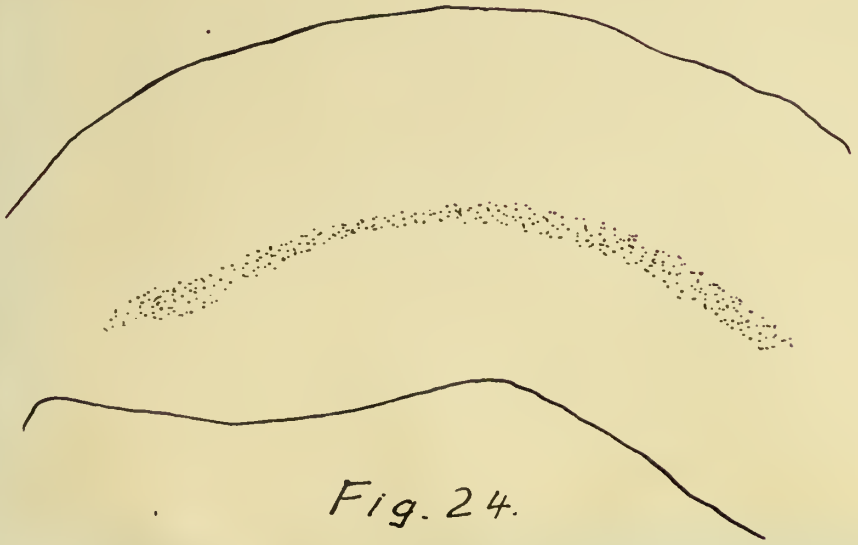

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Fig. 25.

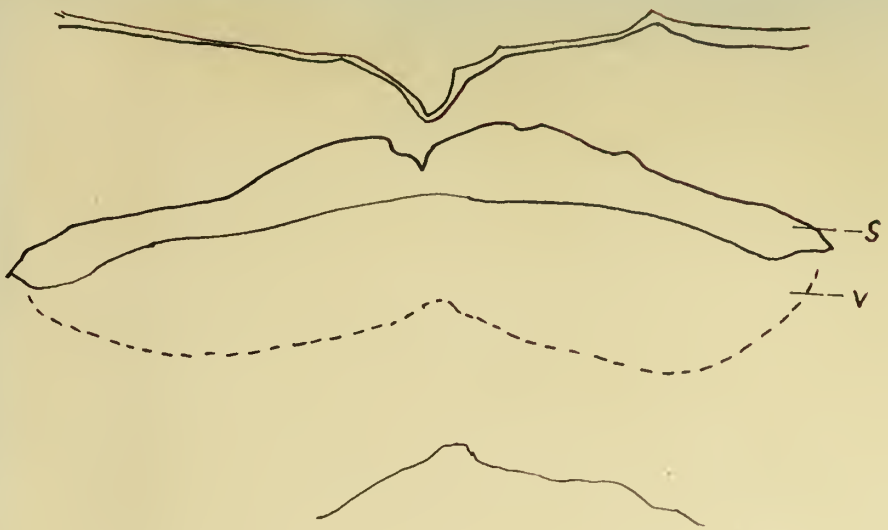
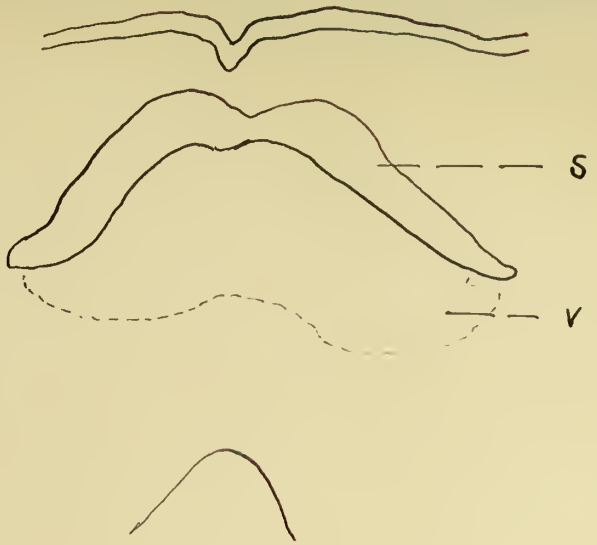


Fig. 26.

Fig. 27.



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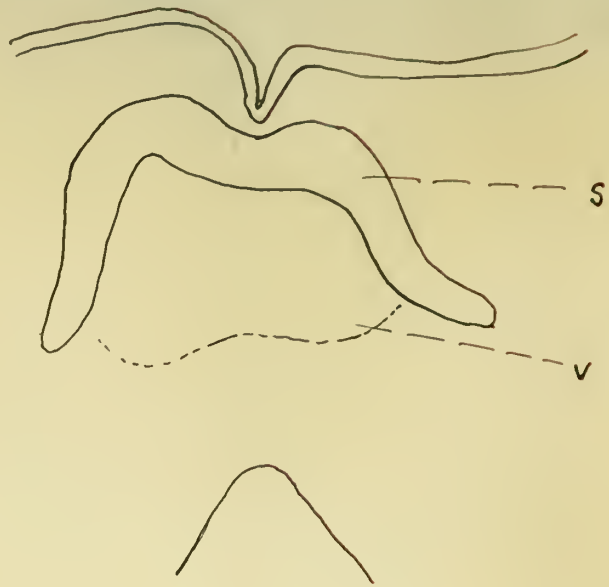

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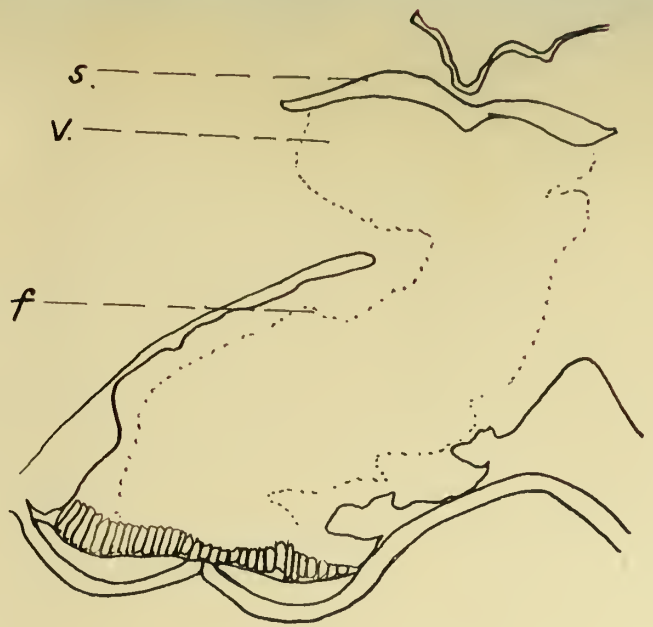

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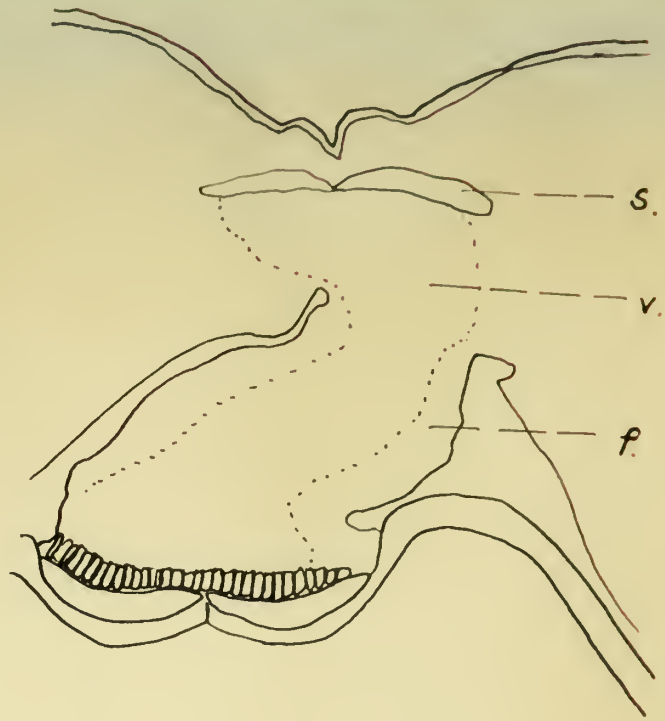

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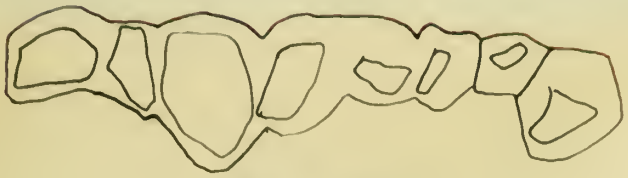

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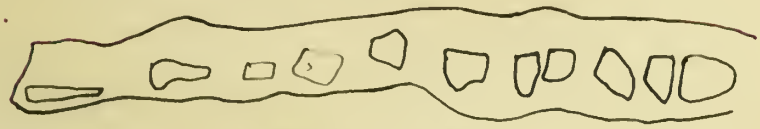

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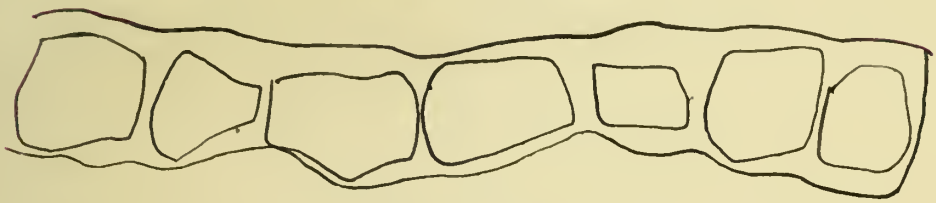

Fig. 34.

Fig. 35.

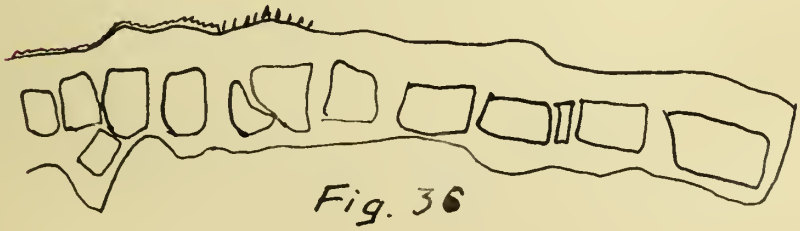

Fig. 36

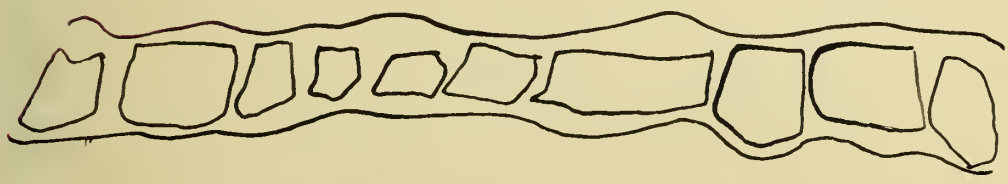

Fig. 37.